

Claims

1. System for replicating or for replicating and expressing a sequence of interest in a plant, comprising:
 - (i) a DNA precursor of an RNA replicon, said RNA replicon being derived from a plus-sense single stranded RNA virus and comprising at least one sequence of interest, said DNA precursor of said RNA replicon containing one or more introns; and
 - (ii) a DNA precursor of a helper replicon, wherein said helper replicon is
 - (a) incapable of systemic movement in a plant both in the presence and in the absence of said RNA replicon (i) and
 - (b) capable of expressing in a plant one or more proteins necessary for systemic movement of said RNA replicon (i),whereby said RNA replicon (i) is capable of replicating or of replicating and expressing said sequence of interest in a plant, but unable to move systemically in a plant in the absence of said one or more proteins expressed by said helper replicon (ii).
2. The system according to claim 1, wherein said helper replicon (ii) is incapable of systemic movement in a plant due to the absence of a functional origin of viral particle assembly.
3. The system according to claim 1 or 2, wherein said helper replicon (ii) is capable of expressing in a plant a coat protein and/or a movement protein necessary or useful for said systemic movement of said RNA replicon (i) in said plant.
4. The system according to any one of claims 1 to 3, wherein said RNA replicon (i) cannot express a protein necessary for systemic movement of said RNA replicon (i) in said plant.
5. The system according to claim 4, wherein said RNA replicon (i) lacks a coat protein open reading frame and said sequence of interest is larger than 1 kb.
6. The system according to any one of claims 1 to 5, wherein said RNA replicon (i) is based on, or contains components of, a tobamovirus.
7. The system according to claim 6, wherein said tobamovirus is a tobacco mosaic virus.

8. The system according to claim 6 or 7, wherein said RNA replicon (i) is based on a tobamovirus wherein the coat protein open reading frame is replaced by said sequence of interest.
9. The system according to any one of claims 1 to 5, wherein said RNA replicon (i) is based on or contains components of alfalfa mosaic virus.
10. The system according to any one of claims 1 to 5, wherein said RNA replicon (i) is based on or contains components of potato virus X.
11. The system according to any one of claims 1 to 5, wherein said RNA replicon (i) is based on or contains components of cowpea mosaic virus.
12. The system according to any one of claims 1 to 11, wherein said precursor of said helper replicon (ii) is DNA encoding said helper replicon (ii), whereby said DNA is capable of producing said helper replicon (ii) in cells of said plant.
13. The system according to any one of claims 1 to 12, wherein said precursor of said RNA replicon (i) or said precursor of said helper replicon (ii) are carried by agrobacteria.
14. The system according to any one of claims 1 to 13, wherein the system further comprises a plant, or seeds thereof, for replicating or for replicating and expressing said sequence of interest.
15. The system according to any one of claims 1 to 14, wherein said plant is a dicot plant, preferably a *Solanacea* plant, more preferably a *Nicotiana* plant, most preferably tobacco.
16. The system according to any one of claims 1 to 15, wherein said plant is transgenic and expresses a viral protein necessary or useful for cell-to-cell movement of said RNA replicon (i) or said helper replicon (ii).
17. The system according to claim 16, wherein said viral protein is a movement protein of tobacco mosaic virus.
18. The system according to any one of claims 1 to 17, wherein said RNA replicon (i)

and said helper replicon (ii) lack homology in functionally overlapping regions.

19. The system according to any one of claims 1 to 18, whereby said RNA replicon (i) and said helper replicon (ii) lack a recombination-prone homology in a region recombination in which between said RNA replicon (i) and said helper replicon (ii) would create an RNA replicon capable of expressing a protein necessary for systemic movement and capable of moving systemically in said plant.
20. The system according to any one of claims 1 to 19, wherein the sequence homology between said RNA replicon (i) and said helper replicon (ii) in any sequence segments having at least 100 nucleotides is at most 80%.
21. The system according to claim 20, wherein said sequence segments are located downstream of the replicase ORFs of said RNA replicon (i) and said helper replicon (ii).
22. The system according to any one of claims 1 to 21, wherein said helper replicon (ii) lacks a movement protein ORF but contains a replicase ORF and an ORF encoding a protein necessary for systemic movement of said RNA replicon (i), the latter ORF being under the control of a subgenomic promoter, said subgenomic promoter being derived from an RNA virus of a strain different from the RNA virus from which the subgenomic promoter controlling expression of said sequence of interest in said RNA replicon (i) is derived.
23. The system according to any one of claims 1 to 22, wherein the sequence of said RNA replicon (i) and the sequence of said helper replicon (ii) do not overlap.
24. The system according to any one of claims 1 to 23, wherein said DNA precursor of said RNA replicon (i) contains one or more introns in the replicase ORF of said RNA replicon (i).
25. A process for replicating or for replicating and expressing a sequence of interest in a plant, comprising providing cells of a plant with
 - (i) a DNA precursor of an RNA replicon, said RNA replicon being derived from a plus-sense single stranded RNA virus and comprising at least one sequence of interest, said DNA precursor of said RNA replicon containing one or more introns; and

- (ii) a DNA precursor of a helper replicon, wherein said helper replicon is
 - (a) incapable of systemic movement in said plant both in the presence and in the absence of said RNA replicon (i) and
 - (b) capable of expressing in a plant one or more proteins necessary for systemic movement of said RNA replicon (i),

whereby said RNA replicon (i) is capable of replicating or replicating and expressing said sequence of interest in said plant, but unable to move systemically in said plant in the absence of said one or more proteins expressed by said helper replicon (ii).

- 26. The process according to claim 25, wherein said plant is provided with said RNA replicon (i) and/or said helper replicon (ii) by transfecting with agrobacteria containing in their T-DNA said precursor of said replicon (i) and/or with agrobacteria containing in their T-DNA said precursor of said helper replicon (ii).
- 27. The process according to any one of claims 25 to 26, wherein a part of said plant like a leaf is provided with said RNA replicon (i) and said helper replicon (ii) but not other parts of said plant.
- 28. The process according to any one of claims 25 to 27, wherein said sequence of interest is capable of replicating or of replicating and expressing systemically in parts of said plant not provided with both said RNA replicon (i) and said helper replicon (ii).
- 29. The process according to any one of claims 25 to 27, wherein said plant is a dicot plant, preferably a *Solanacea* plant, more preferably a *Nicotiana* plant, most preferably said plant is tobacco.
- 30. A process for replicating or for replicating and expressing a sequence of interest in a plant, comprising providing cells of a plant with
 - (i) a DNA precursor encoding an RNA replicon, said RNA replicon being derived from a plus-sense single stranded RNA virus and comprising at least one sequence of interest; and
 - (ii) a DNA precursor encoding a helper replicon, wherein said helper replicon is
 - (a) incapable of systemic movement in said plant both in the presence and in the absence of said RNA replicon (i) and
 - (b) capable of expressing in a plant one or more proteins necessary for systemic movement of said RNA replicon (i),

whereby

said RNA replicon (i) is capable of replicating or replicating and expressing said sequence of interest in said plant, but unable to move systemically in said plant in the absence of said one or more proteins expressed by said helper replicon (ii);

and

said DNA precursor encoding said RNA replicon (i) contains sequences for replicon function of said RNA replicon (i), said sequences being derived from a sequence of said RNA virus, said sequences for replicon function exhibit at selected localities of said sequence of said RNA virus function-conservative differences from said sequence of said RNA virus, said differences causing an increased frequency of RNA (i) replicon formation compared to an RNA replicon not exhibiting said differences.

31. The process according to claim 30, wherein said helper replicon (ii) is derived from a plus-sense single stranded RNA virus and said DNA precursor encoding said helper replicon (ii) contains sequences for replicon function of said helper replicon (ii), said sequences being derived from a sequence of said RNA virus, said sequences for replicon function exhibit at selected localities of said sequence of said RNA virus function-conservative differences from said sequence of said RNA virus, said differences causing an increased frequency of helper replicon formation compared to a helper replicon not exhibiting said differences.
32. The process according to claim 30 or 31, wherein said providing cells of a plant with said RNA replicon (i) and said helper replicon (ii) comprises transient Agrobacterium-mediated transformation with said DNA precursor (i) and said DNA precursor (ii).
33. Use of the system according to any one of claims 1 to 24 for expressing a protein of interest in a plant from said sequence of interest.